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REP05988GB

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04 JAN 1999

9900082.0

3. Full name, address and postcode of the or of each applicant (underline all surnames)

Microscience Ltd.
67-68 Jermyn Street
London
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United Kingdom

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

7304546001

4. Title of the invention

PROTEIN AND COMPOSITIONS CONTAINING IT

5. Name of your agent (if you have one)

GILL JENNINGS & EVERY

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

Broadgate House
7 Eldon Street
London
EC2M 7LH

Patents ADP number (if you know it)

745002

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Date of filing
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Number of earlier application

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Description 7

Claim(s) 1

Abstract

Drawing(s) 1 & 1

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Priority documents

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Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for preliminary examination and search (Patents Form 9/77)

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11. For the Applicant
Gill Jennings & Every

I/We request the grant of a patent on the basis of this application.

Signature

Date

4 January 1999

12. Name and daytime telephone number of person to contact in the United Kingdom

PERRY, Robert Edward
0171 377 1377

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PROTEIN AND COMPOSITIONS CONTAINING IT

Field of the Invention

This invention relates to one protein, to vaccines
5 containing it, and to its use in therapy, for
immunisation.

Background to the Invention

Group B Streptococcus (GBS), also known as
Streptococcus agalactiae, is the causative agent of
10 various conditions. In particular, GBS causes:

Early onset neonatal infection.

This infection usually begins *in utero* and causes
severe septicaemia and pneumonia in infants, which is
lethal if untreated and even with treatment is associated
15 with a 10-20% mortality rate.

Late onset neonatal infection.

This infection occurs in the period shortly after
birth until about 3 months of age. It causes a
septicaemia, which is complicated by meningitis in 90% of
20 cases. Other focal infections also occur including
osteomyelitis, septic arthritis, abscesses and
endophthalmitis.

Adult infections.

These appear to be increasingly common and occur
25 most commonly in women who have just delivered a baby,
the elderly and the immunocompromised. They are
characterised by septicaemia and focal infections
including osteomyelitis, septic arthritis, abscesses and
endophthalmitis.

30 *Urinary tract infections.*

GBS is a cause of urinary tract infections and in
pregnancy accounts for about 10% of all infections.

Veterinary infections.

GBS causes chronic mastitis in cows. This, in turn, leads to reduced milk production and is therefore of considerable economic importance.

5 GBS infections can be treated with antibiotics. However, immunisation is preferable. It is therefore desirable to develop an immunogen that could be used in a therapeutically-effective vaccine.

Summary of the Invention

10 According to the present invention, a partial GBS gene sequence, pho3-23, has been found which represents a probable outer surface protein with unknown function.

In one aspect of the invention, the use of this protein in a recombinant protein vaccine is described.
15 This vaccine may be administered to females either prior to, or during pregnancy to protect mother and neonate against infection by GBS.

The gene sequence may be first genetically altered to increase the antigenicity of the encoded protein.

20 Brief Description of the Drawings

The invention will now be described in detail with reference to the accompanying figures, wherein:

Figure 1 shows the nucleotide sequence of the insert of clone pho3-23 and the deduced amino acid sequence of
25 ORF3-23.

Description of the Invention

Because of its extracellular or cell surface location, the protein of the present invention may be a suitable candidate for the production of therapeutically-effective vaccines against GBS.
30 The term "therapeutically-effective" is intended to include the prophylactic effect of the vaccines. For example, a recombinant protein may be used, as an antigen for direct administration to a patient. The protein may be isolated

directly from GBS expressed in any suitable expression system, e.g. *Lactococcus lactis*. It is preferably administered with an adjuvant, e.g. alum.

5 The protein may be a mutant protein, in comparison to wild-type protein, a fragment of the protein or a combination of different fragments, provided an effective immune response is generated.

10 An alternative approach is to use a live attenuated GBS vaccine. This may be produced by deleting the gene that encodes the protein. Preferably, the GBS strain comprises additional virulence gene mutations.

The protein (or fragments thereof) of the present invention may also be used to produce monoclonal and polyclonal antibodies for use in passive immunisation.

15 In a further embodiment of the invention, the protein or corresponding polynucleotide may be used as a target for screening potentially useful drugs, especially antimicrobials. Suitable drugs may be selected for their ability to bind to the protein to exert their effects.
20 Assays for screening for suitable drugs and which make use of the protein of the invention will be apparent to those skilled in the art.

Although the protein has been described for use in the treatment of patients, veterinary uses of the protein
25 are also considered to be within the scope of the present invention. In particular, the protein or the vaccines may be used in the treatment of chronic mastitis, especially in cows.

The present invention is described with reference to
30 Group B Streptococcal strain M732. However, all the GBS strains and many other bacterial strains are likely to include related proteins having amino acid sequence homology with the protein of M732. Organisms likely to contain the proteins include, but are not limited to, *S.*

pneumoniae, *S. pyogenes*, *S. suis*, *S. milleri*, Group C and Group G *Streptococci* and *Enterococci*. Vaccines to each of these may be developed in the same way as described for GBS.

5 Preferably, the proteins that may be useful for the production of vaccines have greater than 40% sequence similarity with the protein of M732. More preferably, the proteins have greater than 60% sequence similarity. Most preferably, the proteins have greater than 80%
10 sequence similarity.

 The protein of the present invention was identified as follows:

 A partial gene library of GBS (strain M732) chromosomal DNA was prepared using the plasmid vectors
15 pFW-*phoA*1, pFW-*phoA*2 and pFW-*phoA*3 (Podbielski, A. et al. 1996. Gene 177:137-147). These plasmids possess a constitutive spectinomycin adenyltransferase antibiotic resistance marker, which confers a high level of spectinomycin resistance and is therefore easily
20 selected. Furthermore, these vectors contain a truncated (leaderless) *Escherichia coli phoA* gene for alkaline phosphatase. The three vectors differ only with respect to the reading frame in which the leaderless *phoA* gene exists, as compared to an upstream in-frame *Bam*HI
25 restriction enzyme site. Because this truncated *E. coli phoA* gene lacks the appropriate leader sequence for export of this enzyme across the bacterial membrane, extracellular alkaline phosphatase activity is absent when these plasmids are propagated in an *E. coli phoA*
30 mutant (e.g. strain DH5 α). The chromogenic alkaline phosphatase substrate, XP (5-Bromo-4-chloro-3-indolyl-phosphate), does not enter intact bacterial cells and therefore only exported or surface associated alkaline phosphatase activity can be detected. When exported or

surface associated alkaline phosphatase activity is present, the chromogenic XP substrate is cleaved to yield a blue pigment and the corresponding bacterial colonies can be identified by their blue colour.

5 Plasmid DNA was digested to completion with *Bam*HI and dephosphorylated using shrimp alkaline phosphatase. GBS genomic DNA was partially digested with *Sau*3AI, size fractionated on a sucrose gradient and fragments <1kb in size were ligated into the prepared pFW-*phoA* vectors. *E.*
 10 *coli* strain DH5 α was chosen as the cloning host since it lacks a functional *phoA* gene. Recombinant plasmids were selected on Luria agar containing 100 μ g/ml of spectinomycin and 40 μ g/ml of the chromogenic XP substrate. *E. coli* transformants harbouring plasmids
 15 containing GBS insert DNA that complements the export signal sequence of the leaderless *phoA* gene were identified by the blue colour of the colonies. Approximately 30000 different recombinant plasmids containing GBS insert DNA were screened in this manner
 20 and 83 recombinant plasmids, which complemented the leaderless *phoA*, were chosen for further study.

From these experiments, one clone was selected containing a plasmid designated *pho3-23*. This plasmid contained a gene (or part thereof), which complemented
 25 the leaderless *phoA*. Plasmid *pho3-23* contained 314 bp of GBS DNA and the nucleotide and deduced amino acid sequences are shown in Figure 1.

A comparison of the amino acid sequence of ORF3-23 was performed and the results are shown in Table 1.

30 As shown in Table 1, homologues to the GBS ORF3-23 gene product can be identified in *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Enterococcus faecalis* and *Streptococcus mutans*. The *S. pyogenes*, *S. pneumoniae* and *E. faecalis* homologues were identified from genome

sequence data and no annotations were available as to the identity of the gene or gene products. In *S. mutans*, the *perM* gene product has been presumptively identified as a permease, but no other information is available as to the function of this protein. Considering that the ORF3-23 coding region complements the leaderless *phoA* gene, it can be concluded that the ORF3-17 gene product would most probably be located extracellularly.

Table 1. Database search results for ORF3-23 (104 amino acids)

| Organism | Protein Accession | DNA Accession | Gene Name | % Similarity | % Identity | Alignment Length |
|----------------------|----------------------|------------------|--------------|-----------------|---------------|---------------------|
| <i>S. pyogenes</i> | bp 35280-36458 | Contig 290 | Unknown | 66.35 | 57.69 | 104 |
| <i>S. pneumoniae</i> | bp 7480-8643 | Contig 4182 | Unknown | 58.65 | 47.11 | 104 |
| <i>E. faecalis</i> | bp 32197-33379 | Contig 6174 | Unknown | 57.69 | 46.15 | 104 |
| <i>S. mutans</i> | TR:O68573 | EM:AF051356 | perm | 42.31 | 32.69 | 105 |

CLAIMS

1. A protein comprising an amino acid sequence encoded by the polynucleotide defined as ORF3-23 in Figure 1 or a homologue thereof with at least 60% sequence homology.
- 5 2. A protein according to claim 1, obtainable from the Group B streptococcal strain M732.
3. A protein according to claim 1 or claim 2, wherein ORF3-23 comprises the nucleotides 2-313.
4. A protein according to any of claims 1 to 3, for use
10 in a method of therapy.
5. A polynucleotide which encodes a protein according to any preceding claim, its complement, or a fragment thereof.
6. The use of a bacterial protein according to any of claims 1 to 4, in the manufacture of a vaccine to treat
15 bacterial infection.
7. The use according to claim 6, wherein the infection is a Group B streptococcal infection.
8. The use according to claim 6 or claim 7, wherein the infection is a focal infection.
- 20 9. The use according to claim 6 or claim 7, wherein the infection is a urinary tract infection.
10. Use of a product according to any of claims 1 to 5, for screening potential antimicrobial drugs.
11. An antimicrobial drug selected using the products as
25 defined in claim 10.
12. A vaccine comprising a product according to any of claims 1 to 5.
13. A vaccine comprising a microorganism having a virulence gene deletion, wherein the gene codes for a
30 protein according to any of claims 1 to 4.
14. An antibody raised against a protein according to any of claims 1 to 4.

Figure 1. Nucleotide and deduced amino acid
sequence of clone pho3-23

START ORF3-23 (truncated)
| 10 30 50
GATCTTAAATAATCAAGCAGTTATTGCTCTCATGATTACCTTTTTGGTAT
I L N N Q A V I A L M I T F L V F

 70 90
TTTTAACGATTTTTATTTTTACCAAATCTCTTTTATGTTTAAACCTGTG
L T I F I F T K I S F M F K P V

 110 130 150
TTTGATTTTCTTGCTGTGCTGATATTGCCGCTTGTAATTTCTGGCTTGCT
F D F L A V L I L P L V I S G L L

 170 190
TTATTACCTATTAAACCTATGGTTACATTTTTAGAGAAGCGGGGAATTA
Y Y L L K P M V T F L E K R G I K

 210 230 250
AGCGTGTAACAGCGATATTATCAGTTTTTACTATTATAATCCTTCTGTTA
R V T A I L S V F T I I I L L L

 270 290
ATTTGGGCAATGTCTAGTTTTATTCCCATGATGAGTAATCAATTACGCCA
I W A M S S F I P M M S N Q L R H

 310
TTTTATGGAAGATC 314
F M E D

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